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INTESTINAL FLORA OF MONKEYS AND DOGS DURING
DIGESTION AND FOLLOWING THE DIRECT INTRO-
DUCTION OF FOOD SUBSTANCES INTO THE
CECUM AND INTO ISOLATED SEGMENTS
OF BOWEL

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HYGIENE AND BACTERIOLOGY
1933

By
ELIZABETH PETRAN

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ELIZABETH PETRAN

CHICAGO

The mucosa of the large intestine makes up a part of the body surface and, like other body surfaces, is in contact with microorganisms, but with larger numbers and different kinds. Here the contents of the bowel, separated from the body tissues only by a single layer of epithelium, maintain a favorable environment for the multiplication of many types of organisms. It is an environment of constant temperature, shut off from the deleterious action of light and subject to the continuous changes accompanying digestion.

The bacteriological investigation of this region is a major problem, beset with many difficulties. The isolation of the many kinds of bacteria present in the contents of the colon is difficult with our present inadequate methods. The bacteria of the colon group will grow on the simplest synthetic mediums and will completely overgrow many other organisms unless selective mediums are employed. Those organisms which belong to the coccus, aciduric, micro-aerophilic and non-sporulating anaerobic groups develop well only on rich mediums and under certain conditions of oxygen tension. There is as yet no satisfactory method for the quantitative estimation of the vegetative cells of the group of spore-forming anaerobic bacteria, unless they happen to be the predominating organisms. Other organisms, such as intestinal spirochetes, have not been cultivated. Some bacteria are so similar in their cultural requirements that it is difficult to separate them. For example, in a mixture of known numbers of *Bact. prodigiosum* and *Bact. coli* it is impossible to detect the presence of one organism if it is greatly outnumbered by the other.¹

Bacteriological relationships in the intestine are influenced by factors, of which some are unknown and others are not adaptable to control. The chemical composition of intestinal contents, about which little is known, may be an important factor in determining the flora. London and his coworkers² in their studies on absorption made extensive chemical analyses of material collected from fistulae at various levels of the intestines of dogs. They found that at least three-fourths of the food is absorbed in the small intestine and that the greater part of the absorption occurs in the upper portion. The

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From the Department of Hygiene and Bacteriology, University of Chicago.

1. Dack, G. M., and Petran, E.: *J. Infect. Dis.* **54**: 204, 1934.

2. *Ztschr. f. physiol. Chem.* **60**: 191, 1909.

menstruum available for bacterial growth in the large intestine is thought to be composed primarily of undigested food, unabsorbed metabolic products, various digestive secretions and products resulting from bacterial enzyme activity. Obviously it is impossible to duplicate in vitro conditions found in the large intestine.

A further obstacle encountered is the inaccessibility of the intestinal content at most levels. An examination of the feces does not necessarily disclose conditions as they exist naturally in other portions of the bowel, nor can material removed at necropsy in experimental animals or man give a true picture of the labile bacterial relationships when peristalsis and secretions are altered or absent. In view of these difficulties, it seems apparent that no single approach to the problem can offer an adequate or final answer to the many questions which await solution in this field.

Means are available, however, by which certain of these difficulties may be partially overcome. By a method previously described,³ surgical fistulae prepared in experimental animals permit access to any desired level of the intestinal tract so that samples may be obtained at will under relatively physiological conditions. The fistulae also provide channels through which various substances may be introduced directly into the respective levels with the view of determining the influence of such substances on the bacterial life. Isolated loops of gut from which the intestinal stream is eliminated are of value in studying bacterial activity influenced only by the secretions and other physiological processes of the particular bowel segment.¹ These two methods have been used in the present study to determine the effect on bacterial activity of altering the nutritional environment in the colon of dogs and monkeys.

LITERATURE

Escherich in 1886⁴ observed that the stools of breast fed infants contained a different flora from that of children or adults on a regular diet; but it was not until 1909 (Herter and Kendall)⁵ that the influence of diet on the intestinal flora was experimentally studied in animals. Monkeys and cats on a high protein diet were found to develop a proteolytic flora which could be changed to a non-proteolytic type by feeding a diet rich in carbohydrates. The association of many pathological conditions in man with putrefactive types of bacteria in the intestine led to an extensive study to determine which food substances were capable of effecting a substitution by a fermentative type of flora. Distaso and Schiller,⁶ Hull and Rettger,⁷ Rettger and Cheplin,⁸ Cannon,⁹ Cruickshank¹⁰ and others have found that lactose or

3. Dack, G. M., and Woolpert, O. C.: *J. Prev. Med.* **6**: 129, 1932.

4. *Darmbakterien des Sauglings*, Stuttgart, 1886 (from Kendall Textbook of Bacteriology).

5. *J. Biol. Chem.* **7**: 203, 1909.

6. *Compt. rend. Soc. de biol.* **76**: 243, 1917.

7. *J. Bact.* **2**: 47, 1917.

8. *A Treatise on the Transformation of the Intestinal Flora*. Yale University Press, New Haven. Rettger, L. F., and Cheplin, H. H. 1921.

9. *J. Infect. Dis.* **29**: 369, 1921.

10. *Brit. J. Exper. Path.* **9**: 318, 1928.

dextrin added in sufficient quantities to the diet of white rats was effective in producing an aciduric type of intestinal flora. Other carbohydrates, such as dextrose, maltose, levulose, galactose and possibly sucrose, produced no change in the bacterial relationships. Torrey¹¹ in experiments with dogs confirmed these observations. He found that a basic diet of rice and beef heart caused the predominance of *Bact. coli*, while a basic diet of bread and beef heart led to the development of an aciduric flora. He thought that the efficacy of the bread in stimulating the development of *L. acidophilus* was due to its content of dextrin.

Various theories have been advanced to explain the change brought about in the intestinal flora by the oral administration of lactose. Kendall¹² and Cannon and McNease¹³ claimed that the carbohydrate was acted upon by both proteolytic and fermentative organisms and that the acidity produced was unfavorable to the former group. Rettger¹⁴ admitted that where the amount of lactose fed was large acidity might play a part, but he stated that the change was due mainly to the fact that a large proportion of carbohydrate reached the colon, where it served as a favorable medium for the growth of *L. acidophilus*. Cruickshank¹⁵ agreed with Kendall and Cannon that lactose fed in large amounts was attacked by *Bact. coli*, *Cl. welchii* and other active carbohydrate fermenters, and that the acid substances produced offered suitable pabulum for the development of *L. acidophilus*.

The effect of high protein diets on the intestinal flora has received little attention in the past. As the literature has been reviewed recently by Torrey and Montu,¹⁶ only the papers pertinent to the present investigation will be mentioned. Herter and Kendall⁵ found that monkeys and cats on a high protein diet were sleepy and slow in motion. The feces inoculated into fermentation tubes containing sugar produced a large amount of gas. They were unable in these experiments to isolate *Cl. welchii* from the intestinal contents of monkeys. Torrey,¹¹ on the other hand reported that in dogs on a beef heart diet *Cl. welchii* was the predominating organism, and that the percentage of *Bact. coli* was reduced below that for a mixed diet. Cannon⁹ reported practically the same finding in white rats fed on a diet of ground beef. Sanborn¹⁷ investigated the effect of diets high in protein on the fecal flora of six persons. She found that an increase in the numbers of *Bact. coli* occurred in all but two individuals, in whose feces this organism already predominated. There also occurred an increase in sporulating anaerobes in the flora of some of the persons. Torrey and Montu¹⁶ made an extensive study of the fecal bacteria of two arctic explorers who were on an exclusive meat diet for over a year, and of one person who remained on a similar diet for 10 days. A decrease was found in total bacterial numbers. This was due chiefly to the

11. J. M. Research. **39**: 415, 1919.

12. Textbook of Bacteriology, 625, 1921.

13. J. Infect. Dis. **32**: 175, 1923.

14. Am. J. Pub. Health **19**: 771, 1929.

15. Brit. M. J. **2**: 555, 1928.

16. J. Infect. Dis. **49**: 141, 1931.

17. J. Infect. Dis. **49**: 37, 1931.

suppression of *L. acidophilus*, *B. bifidus*, enterococci, streptococci and a group of micro-aerophilic bacteria. In one subject there was a marked decrease in the numbers of viable *Bact. coli*. During the first months on the diet there was an increase in the numbers of *Cl. welchii*, which was reduced to normal levels after one year.

In the investigation reported here intensive studies have been carried out on a few animals in order to determine the normal relationships of bacteria in the colon and the effect on these relationships of changing the nutritional environment. By the addition of nutrient materials attempts were made to alter the bacterial relationships in the rather simplified flora of isolated segments of colon in two monkeys.

MATERIALS AND METHODS

Three dogs and one monkey with cecal fistulae, one monkey with an ileal fistula, two monkeys with isolated segments of large intestine, and one dog with a biliary fistula were used in this study. The surgical preparation of the latter animal is described below. The preparation of the other animals, which had been used in previous experiments, is described in detail elsewhere.¹ The significant features for the purposes of the present study may be briefly recounted.

At different levels of the bowel non-leaking fistulae have been formed which permit the direct introduction of food or other material into the respective level, or the removal of bowel content from that level, repeatedly and at will, with the minimal physiological disturbance.

These fistulae are merely small segments of the living gut itself, one end of the segment having been brought out to the body surface, the other end opening into the intestine. They do not leak because the proximal end of the bowel segment is outward so that peristalsis within the loop tends to carry materials inward, keeping the segment relatively free from bacteria. After the animal has recovered from the operation such fistulae do not constitute a serious disturbance to intestinal functions. The segments are short, their mesenterical attachments are intact, and only the healthy intestinal mucosa is exposed at the orifice. Such animals have been kept many months in apparently perfect health.

The isolated loops of bowel are simply short segments which have been excised from the intact gut and of which both ends are brought to the body surface, the continuity of the gut being re-established by end-to-end anastomosis. They permit the exclusion of the many complex factors associated with the continuous stream of intestinal content, yet include all of the physiological activities associated with the particular bowel segment apart from those which might be dependent on the normal bowel content for their motivation.

Intestinal contents were readily aspirated through sterilized rubber catheters introduced into the fistulae, the orifices of which had been previously sponged with a dry sterile cotton plug. The use of antiseptics or disinfectants to sterilize the orifices was not practical, as any effective disinfectant would injure the intestinal mucosa. Material could be aspirated from the ascending colon at any time between 1 and 12 hours after feeding, but with difficulty or not at all after a longer interval.

As the isolated segments of bowel contained no appreciable amount of material which could be aspirated for culturing, some dilution substance had to be introduced. For this purpose a sterile bread and milk paste was used in some experiments and saline in others. When the former was used samples

were taken 30 minutes after injection to allow natural mixing due to bowel motility and at the same time to avoid multiplication of bacteria in the injected pabulum.

Quantitative decimal dilutions of the specimens were made in sterile 0.85 per cent NaCl solution. This dilution was great enough (1:100 billion) to permit an estimation of the total viable organisms.

In this investigation whichever organism outnumbered all others, as determined by the technic described below, was isolated and compared with the numbers of *Bact. coli* and in some experiments with the numbers of streptococci, *L. acidophilus* and spore-forming anaerobes.

Lactose broth fermentation tubes, rather than a solid medium, were used for the quantitative estimation of *Bact. coli* because in previous experiments¹ where a comparative study was made, using lactose broth tubes and plates of Ayers-Rupp synthetic agar,¹⁸ higher counts were obtained with the former method. These results are in agreement with those of Sanborn¹⁹ who found in enumerating groups of fecal organisms that the highest total counts were obtained in broth mediums. Tubes of lactose broth were inoculated with 1 cc. of each dilution of the specimen and incubated at 37 C. for 24 to 48 hours. The numbers of *Bact. coli* were determined by streaking the positive lactose broth cultures on eosin methylene blue or Endo plates and examining these after 24 hours' incubation for colonies fermenting lactose and having a characteristic metallic luster.

Torrey's glucose liver agar,²⁰ adjusted to p_H 5.0 with acetic acid, was used for identifying *L. acidophilus*. Poured plates were made from 3 to 4 dilutions chosen to give isolated colonies. The colonies of *L. acidophilus* were recognized by their wooly appearance. Certain of these were stained by Gram's method and transferred to azolitmin milk tubes as a further means of identification.

Deep milk tubes and tubes of beef heart medium, with and without dextrose, were used for the detection of the spore-forming anaerobes. These mediums were inoculated with 0.1 cc. of dilutions made from heated (80 C. for 10 minutes) specimens and incubated in anaerobic jars for 4 or 5 days. The milk tubes were then examined for the stormy fermentation typical of *Cl. welchii* and the beef heart cultures were streaked on blood agar plates. Organisms isolated on the blood agar plates, incubated anaerobically, were inoculated into tubes of deep milk and beef heart, and those not producing stormy fermentation were saved for further study.

Streptococci were first enumerated on blood agar pour plates, but in later experiments beef heart medium was substituted, since higher counts were obtained in the latter medium.

One per cent dextrose beef heart medium, p_H 7.6, was used to detect the predominating organism in intestinal contents. This medium was selected because it supports the growth of aerobic and anaerobic cocci, the aciduric

18. J. Bact. 3: 433, 1918.

19. J. Infect. Dis. 48: 541, 1931.

20. J. Bact. 2: 435, 1917.

group, the spore-forming anaerobes, the colon group, and non-sporulating anaerobic rods (*Bacteroides*). In addition, this medium was sometimes used to estimate the numbers of bacteria that were not predominating. The beef heart medium was inoculated with 1 cc. of each dilution of the specimen. The cultures were incubated at 37 C. for 48 hours and then kept at room temperature for 2 weeks to allow time for the development of the slowly growing bacteria. The predominating organism was isolated after 48 hours' incubation by streaking duplicate blood agar plates from the last tube in the series of beef heart medium in which growth occurred. One plate was incubated aerobically for 24 to 48 hours and the other anaerobically for 48 to 72 hours. No attempt was made to identify all of the organisms isolated. *Cl. welchii* was differentiated from other anaerobic gram positive rods by inoculating freshly boiled tubes of milk with the culture in question and examining for stormy fermentation after 2 days' incubation in an anaerobic jar. The gram negative rods were often not *Bact. coli*, as reactions for the latter organisms were not obtained by inoculation of lactose broth tubes and Endo or eosin methylene blue plates. The positive beef heart cultures containing the higher dilutions of the specimen were streaked on Endo or eosin methylene blue plates to determine the presence of *Bact. coli*, as sometimes higher counts were obtained in this medium than in lactose broth inoculated with the same series of dilutions. *L. acidophilus* was frequently isolated in higher dilutions from the beef heart cultures than by direct plating in Torrey's medium.

NORMAL BACTERIAL RELATIONSHIPS IN THE ASCENDING COLON OF DOGS AND MONKEYS

The ascending colon, with its sluggish motility, semi-fluid contents and food residue, offers favorable conditions for the growth and multiplication of certain bacteria.

Contents of the small intestine enter the cecum more or less continuously during the course of digestion. This material, carrying with it exogenous as well as endogenous organisms, becomes heavily inoculated with fecal bacteria as it comes through the terminal ileum. Further inoculation occurs in the ascending colon. Alvarez²¹ believes that there are mass movements in the colon at various times during the day, as well as shallow reverse waves which run over this portion of the gut. This motility in the large intestine doubtless plays a part in mixing the bacteria of the colon with the material from the small intestine.

Although it is known that many types of foreign bacteria must enter the intestine with food, simple methods have not been developed for the detection of many of these exogenous bacteria. In this study, *Bact. prodigiosum* has been used as a suggestive index of the progress and fate of such organisms in the intestine. When introduced with the food under controlled conditions, it could be readily identified at any of the intestinal levels where it appeared in sufficient numbers.

Three dogs having cecal fistulae and two monkeys, one with a terminal

21. *The Mechanics of the Digestive Tract*, New York, Paul B. Hoeber, Inc., 1928.

ileum fistula and the other with a cecal fistula, were used in these experiments. The dogs were on a diet of cooked ground meat, bread and McCollum's salt mixture.²² A suspension of *Bact. prodigiosum*, consisting of saline washings of a 12 hour dextrose agar plate culture, was thoroughly mixed with the food of these animals before each experiment. Material (2 to 3 cc.) for bacteriological examination was collected from the ascending colon at definite intervals after feeding and cultured as already described (table 1). Streptococci were present in the lactose broth tubes, many times in higher dilutions than *Bact. coli*. The numbers of streptococci growing in this medium were frequently higher than those appearing on blood agar pour plates. The highest count obtained for the streptococci was used.

The entrance into the colon of contents from the small bowel did not appreciably affect the numbers of *Bact. coli*; in fact, there was a striking uniformity in the numbers of this organism in different specimens taken from the same animal during digestion. There apparently was an increase in the numbers of streptococci in the contents of the ascending colon of one of the dogs at the 6 and 12 hour intervals (table 1, dog 1, exp. 2) and of another

TABLE 1.—*Bacterial Relationships in the Ascending Colon of Dogs During the Digestive Period*

Dog	Hour of collecting specimen after feeding	p_H of specimen	Numbers in millions per cc. in intestinal contents of			
			<i>Bact. coli</i>	Streptococci	<i>L. acidophilus</i>	<i>Bact. prodigiosum</i>
1	1	7.8	2	20	.02	20
	3	7.4	20	.02	0.2	.02
	6	6.2	20	12	.02	2
	12	—	2000	24	0.2	.00
Exp. II	1	7.0	2	20	.00	20
	6	6.9	20	2000	.00	2
	12	6.7	20	20000	.00	.2
2	1	7.4	.2	.2	2	2
	3	7.0	2	20	24	2
	6	7.6	20	2000	39	.00
	12	7.0	2	20	20	.00
Exp. II	Before feeding	7.2	2	20	20	.00
	1	6.85	2	20	.02	.2
	6	6.6	2	20	80	.2
	12	6.6	.2	20	10	.00
3	1	8.2	.2	20	.00	2
	3	7.6	.2	2	.00	2
	6	7.4	200	20	.00	2
	12	7.0	2	200	.00	.002
Exp. II	1	7.15	.2	.02	.00	2
	6	7.2	20	.02	.00	2
	12	7.65	.2	.00	.2	.002

at the 6 hour interval (table 1, dog 2, exp. 1). Since the mouth and throat normally contain large numbers of streptococci, it is difficult to evaluate their significance in the colon. *Bact. prodigiosum* was present in large numbers in specimens collected 1 to 6 hours after feeding, but by the 12 hour interval it was either present only in low dilutions or was not found. Large numbers of *L. acidophilus* were consistently present in the specimens from one dog, while very few were found in those of the other animals. The p_H of the specimens

22. McCollum, E. V.: J. A. M. A. 48: 1379, 1917.

varied with the animal and the length of time after feeding, but they were usually more acid at the 6 and 12 hour intervals.

Although the actual concentration of organisms in the ascending colon did not appear to increase during digestion, it cannot be concluded that there was no bacterial multiplication taking place. Bacterial proliferation may have been obscured by the dilution of the colon content by secretions and by the incoming material from the small bowel. The more significant fact is that bacterial relationships did not alter markedly during this period when, it is reasonable to assume, the nutritional environment in the colon must have been undergoing modification due to the advent of new material from the ileum.

A study was made of the relationships of organisms in the ascending colon of one monkey (C) and the terminal ileum of another (I) for comparison with the findings on the dogs. The regular diet of these monkeys consisted of bread, milk, and bananas. Specimens of contents of the ascending colon of monkey C were collected 3 to 4 hours after feeding, on 3 different days during a period of 3 months. In 2 of these samples an anaerobic gram positive pleomorphic coccus, and in one an aerobic pleomorphic green-producing streptococcus, were from 1000 to 100 times more numerous than *Bact. coli*. Specimens from this animal consistently had very few *Bact. coli* in relation to other organisms.

In specimens from the terminal ileum of monkey I the relationship of *Bact. coli* to other organisms was quite different from that found in monkey C, although these animals were on the same diet. It has been shown that material in the terminal ileum represents regurgitated cecal contents.¹ In one specimen of ileal contents collected 3 hours after feeding, *Bact. coli*, a non-hemolytic gram positive coccus and *Cl. welchii* were present in equal numbers. In 2 other specimens obtained on different days streptococci were 10 times as numerous as *Bact. coli*.

These results are in agreement with the findings of Sanborn¹⁹ who reported a certain degree of constancy in the relationships of *Bact. coli*, streptococci, and *L. acidophilus* to each other in repeated analyses of fecal specimens from the same person, although there were wide variations among different individuals on the same diet. She attributed these variations to the "metabolic characteristics" of the individual. The frequency with which streptococci outnumber other organisms in the contents of the large intestine is further substantiated by the findings of this same investigator, who used similar methods for examining feces from man.

THE EFFECT OF STARVATION AND CARTHARSIS ON BACTERIAL RELATIONSHIPS IN THE ASCENDING COLON OF DOGS

Although it is known that the intestine cannot be freed from bacteria, it was felt that if the number of bacteria were reduced a more accurate picture of bacterial relationships during digestion could be obtained. Sisson²³ reports that starvation reduces the numbers of organisms in the small intestine

23. Am. J. Dis. Child. 13: 117, 1917.

in proportion to the amount of food withheld and the time of starvation. Kendall²⁴ believes that intestinal antiseptics reduce the numbers of organisms merely by mechanical removal due to the diarrhea produced.

In one experiment the large intestines of three dogs were washed with several liters of warm tap water 12 hours after feeding; these animals were then fasted 36 hours before specimens of intestinal contents were aspirated. Following this sampling, the dogs were fed their regular meal and specimens were collected from the ascending colon 1, 6 and 12 hours later. A significant reduction in the numbers of *Bact. coli* occurred only in dog 1, followed by a slight increase 12 hours after feeding. Several months later another attempt was made to reduce the numbers of organisms in the large intestine (table 2). In this experiment the dogs were given large doses (50 grams) of $MgSO_4$ 6 hours after feeding and were fasted 65 hours before the control specimens were taken. The animals were then fed and material was aspirated from the ascending colon 1, 6, 12 and 24 hours later (table 2). In dogs 1 and 2 there was a slight increase in the numbers of *Bact. coli* up to the 12 hour interval. Cocci exceeded other organisms in 7 of the 10 specimens from dogs 1 and 3, possibly because of the mechanical removal of many organisms by the $MgSO_4$, giving the cocci carried down from higher levels opportunity for survival or multiplication. *Bact. coli* outnumbered all other organisms in the specimens from dog 2. This may have been due to the killing of bacteria which passed through the stomach by the high acidity which may have obtained in this one dog while fasting.

These results indicate that, even where the intestinal content has been greatly reduced by artificial means, there is no marked upset of bacterial equilibrium in the colon following the introduction of new food materials by the natural route.

THE EFFECT OF INTRACECAL INJECTIONS OF DEXTROSE, LACTOSE, PEPTONE AND BILE ON THE FLORA OF THE ASCENDING COLON

The numbers and relationships of organisms in the ascending colon were not appreciably changed by dilution with contents of the small bowel, entering during digestion. *Bact. coli*, although frequently outnumbered by other organisms, was present in uniformly large numbers. However, it is known that ingested food material is largely altered and absorbed before the intestinal stream reaches the ileocecal sphincter. Therefore, the only obvious way of testing the immediate effect of food substances on the flora of the colon is to introduce them directly into this level of the bowel. In the experiments reported here concentrated solutions of dextrose, lactose, peptone and bile were injected directly into the ascending colon through cecal fistulae (table 2).

Food substances introduced in this manner caused little if any change in the bacterial populations of the ascending colon. The intracecal injection of concentrated peptone solution seemed to favor slightly the cocci and the anaerobic gram positive rods. This was particularly true in one experiment in

24. J. M. Research 25: 117, 1911.

TABLE 2.—*Bacterial Populations in the Ascending Colon of Dogs During Digestion and After Intracecal Injections of Peptone, Dextrose, Lactose and Bile*

Dog	Injected intra- ceally with*	Hour of col- lecting speci- men after feeding	Numbers in millions in contents of ascending colon of				pH	
			In lactose broth		In beef heart medium Predominating organism			
			Bact. prodigiosum	Bact. coli	Number	Organism		
1	—	6	.02	2	2	Gram +cocci No hemol.		
	—	6	Not fed	200	20	Bact. coli	6.4	
	MgSO ₄ given orally 65 hours before feeding	Before feeding	—	2	2,000	Green strep.	7.25	
		1	20	200	20	Strep.	6.6	
		6	—	20	2,000	Gram. neg. rod**	5.75	
		12	—	2000	2,000	Green strep.	5.3	
		24	—	22	20	Gram neg. cocci	6.85	
	5 cc. of 50% peptone 6 hours after feeding	6	.002	.002	20	Green strep.	6.37	
		12	—	.002	20	Gram +cocci	5.5	
		18	—	.002	2,000	Gram neg. cocci	5.7	
	5 cc. of 50% peptone daily for 6 days	Before feeding	—	2	2,000	Strep.		
		6	—	2	20,000	Green strep.		
		12	—	200	20,000	Gram +cocci		
	2 cc. of 50% peptone at half hour intervals for 18 hours	Before feeding	—	2	20,000	Pleo. gram + No hemol.		
		3	—	200	200	Cocco bacilli	6.35	
		6	.02	20	200	Gram +rod An.	6.85	
		12	—	20	200	Cl. welchii	6.5	
		18	—	2	200	Gram +cocco bacilli	7.0	
					2,000	Strep. S	7.0	
	5 cc. of 50% dex- trose after feed- ing	$\frac{1}{2}$	—	.02	2,000	Gram +diplococci		
		6	.02	.2	20	Gram +cocci		
	5 cc. of 50% dex- trose daily for 6 days	12	—	.2	20,000	Strep.		
		6	—	20	20	Bact. coli	7.0	
	10 cc. of 50% dextrose daily for 6 days	6	—	2	20	Green strep.		
	10 cc. of 50% lactose daily for 6 days***	3	Not fed	200	2,000	Bact. coli	7.2	
	10 cc. of 50% bile twice a day for 3 days	6	—	20	2,000	Green strep.	6.5	
	2	—	6	.002	20	200	Bact. coli	
		—	6	Not fed	20	200	Bact. coli and Green strep.	6.05
MgSO ₄ given 65 hours before feeding		Before feeding	.02	2	20	Gram +diplococci An.		
		1	2	2	200	No hemol.	6.8	
		6	—	2000	2,000	Gram. neg. rods**	6.6	
		12	—	2000	200	Bact. coli	5.4	
		24	.02	200	200	Bact. coli	5.5	
5 cc. of 50% peptone 6 hours after feeding		6	—	2	20	Gram +rods A	6.15	
		12	—	20	200	Diphtheroid S	5.98	
		18	.002	20	20,000	Strep. No hemol.	6.25	
5 cc. of 50% peptone daily for 6 days		Before feeding	—	200	2,000	Gram neg. rods**		
		6	—	20	20,000	Green strep.		
		12	—	2	20	Gram neg. rods**		
2 cc. of 50% peptone at half hour intervals for 18 hours		Before feeding	—	2	2	Gram +rods An.		
		3	.002	20	2,000	Bact. coli	6.5	
		6	.2	200	2,000	Gram +cocci S	6.7	
		12	—	2	2,000	Cl. welchii	6.5	
		18	—	2	200	Gram +rods An.	6.67	
					200	Not determined	6.98	
5 cc. of 50% dextrose after feeding		$\frac{1}{2}$	20	2	20	Strep.		
	6	2	2	2	Bact. prodigiosum			
	12	—	2	20	Cl. welchii			

TABLE 2.—Continued

Dog	Injected intra- ceally with*	Hour of col- lecting speci- men after feeding	Numbers in millions in contents of ascending colon of				pH
			In lactose broth		In beef heart medium Predominating organism		
			Bact. prodigiosum	Bact. coli	Number	Organism	
2	5 cc. of 50% dextrose daily for 6 days	6	.02	20	200	Gram +cocci Hemol. An.	6.9
	10 cc. of 50% dextrose daily for 6 days	6	.02	200	20	Green strep. Bact. coli	6.4
	10 cc. of 50% lactose daily for 6 days***	6	Not fed	20	200	Bact. coli	6.4
	10 cc. of 50% bile twice a day for 3 days	6	20	20	2,000	Gram neg. rods**	6.35
	—	6	—	20	200	Cl. welchii	
	—	6	Not fed	20	20	Bact. coli	6.35
3	MgSO ₄ given 65 hours before feeding	Before feeding	—	.2	20	Green strep.	6.5
		1	20	200	20	Bact. prodigiosum	6.4
		6	.2	2	20	Green strep. and Bact. coli	5.85
		12	—	20	2,000	Green strep.	6.05
		24	—	.2	20	Green strep.	6.25
	5 cc. of 50% peptone 6 hours after feeding	6	.02	.02	200	Strep.	6.5
		12	—	.2	200	Gram +rods S	6.48
		18	—	.02	200	Strep.	7.55
	5 cc. of 50% peptone daily for 6 days	Before feeding	—	.2	2,000	Gram +cocci No hemol.	
		6	.02	.02	20	Cl. welchii	
		12	.02	.02	20	Green pleo. cocci	
	2 cc. of 50% peptone at half hour intervals for 18 hours	Before feeding	—	200	2	Bact. coli and Gram +rod An.	7.5
3		.002	200	200	Gram +rods An.	6.75	
6		.002	2	2,000	Cl. welchii	6.45	
12		—	20	200	Gram +rods and Strep. S	6.8	
18		—	2	200	(Not determined)	7.2	
5 cc. of 50% dextrose after feeding	3	.2	2	2,000	Green strep.		
	6	.2	20	2,000	Gram +cocci An.		
		12	—	2	20,000	Pleo. strep.	
5 cc. of 50% dextrose daily for 6 days	6	.2	2	2	Gram +cocci and Bact. coli		
10 cc. of 50% dextrose daily for 6 days	6	.2	20	200	Green strep.	6.85	
10 cc. of 50% lactose daily for 6 days***	6	Not fed	200	20	Bact. coli Hemol.	8.0	
10 cc. of 50% bile twice a day for 3 days	6	20	20	200	Cl. welchii and Gram +cocci	7.2	

* Unless otherwise noted, injections were made immediately after feeding.

** Not Bact. coli or Bact. prodigiosum.

*** 6 hours after feeding.

An. = Anaerobic growth only. A = Aerobic growth only. S = Present in stained film but did not grow when subcultured. Hemol. = Hemolysis on blood agar plates. No hemol. = No hemolysis on blood agar plates.

which an attempt was made to simulate normal physiological conditions by injecting small amounts of the material at frequent intervals. Two cubic centimeters of 50 per cent peptone were injected intraceally at half hour intervals over a period of 18 hours. Bact. coli was equal in numbers to the predominating organism (table 2) in only the 3 hour culture from 2 of the 3 dogs. In 7 out of 12 cultures made during this experiment, gram positive

anaerobic rods were isolated or seen in a smear from the last tube of beef heart medium in the series in which growth occurred. Three of these produced in milk the stormy fermentation typical of *Cl. welchii*. Some of these gram positive anaerobic rods probably belonged to the genus *Bacteroides*. Sanborn,¹⁹ Torrey and Montu,¹⁶ and Eggerth and Gagnon²⁵ have observed that non-spore-forming anaerobic rods or micro-aerophiles are frequently the predominating organisms in the stools of adults.

Bile, although increasing peristalsis, had no apparent effect on the bacterial flora.

The daily injection of dextrose and lactose may have favored the growth of *Bact. coli*. However, the changes were too slight to permit definite conclusions to be drawn. The intracecal injection of lactose was not followed by an increase in *L. acidophilus*, such as occurred when lactose was fed.¹¹ The question is raised as to the validity of the theory (Rettger's previously stated) that the effectiveness of lactose in producing an aciduric flora depends upon its slow hydrolysis in the intestine and the fact that large amounts reach the cecum in an unaltered state. Although methods were not used in this experiment to detect *L. acidophilus* if it was present in small numbers, its presence may be assumed since the dogs were on the same diet as they were when this organism was found several months before (table 1). In one of the animals it was the predominating organism at this time.

It is interesting to note that the number of *Bact. coli* equaled or exceeded that of any other organism in cecal contents in only 28 per cent of the specimens. There was great variation in the predominating organism in the same animal and even in a series of specimens taken the same day. In a total of 75 cultures made at various intervals during the digestive process and after the injection of different food materials, cocci predominated 34 times (46.5 per cent), *Bact. coli* and anaerobic gram positive rods 10 times each (13.7 per cent), and other organisms less frequently.

Experiments were made on two monkeys for comparison with similar studies on the dogs. One monkey (I) had a fistula into the terminal ileum and the other (C) a cecal fistula. These experiments are presented in tabular form (table 3).

After the animals had been fed an exclusive meat diet for one week, there was a marked change in the relationships of organisms in the colon of monkey C but not in the ileal contents of monkey I. In the former case large gram positive spore-forming anaerobic rods (not *Cl. welchii*) became the predominating organisms, and were still outnumbering other bacteria one week after the regular diet had been resumed. There was also an increase in the numbers of *Cl. welchii* and *Bact. coli* during this period. Both animals had a severe diarrhea while on the diet of autoclaved liver, but when put back on the regular ration this condition promptly subsided. Some cases of infant diarrhea²⁶ have been associated with an increased number of *Cl. welchii* in

25. J. Bact. **25**: 389, 1933.

26. Klein, E.: Ann. Rep. Med. Off. Local Gov. Board, London, No. 27, 210, 1897; Kendall, A. I.: Boston M. & S. J. **169**: 754, 1913; Nelson, C. I.: J. Infect. Dis. **52**: 89, 1933; Tissier, H.: Ann. l'Inst. Pasteur **19**: 273, 1905.

TABLE 3.—*The Effect on the Intestinal Flora in Monkeys of Feeding Lactose and Autoclaved Liver, and the Injection of Lactose into Lower Levels of the Bowel*

Monkey I (fistula into the terminal ileum)								
Date	Preparation	Hour of collecting specimen after feeding	pH of specimen	Numbers in millions per cc. of intestinal contents of				Predominating organism
				Bact. coli	Streptococci	L. acidophilus	Spore-forming anaerobes	
1933								
1/12	Fed regular meal	3	7.0	100	100	Not tested	100	Cl. welchii, streptococci non-hemolytic Bact. coli
1/20	Fed 300 cc. of autoclaved liver daily for 8 days (diarrhea). Specimen collected 8th day.	3	6.8	.1	10	Not found ¹	Not found ¹	Gram negative-pleomorphic coccus
1/27	Regular diet for 8 days. (Stools well formed).	3	8.0	1	10	Not found ¹	Not found ¹	Diplostreptococci ²
2/4	Fed 50 grams lactose daily for 8 days ³ (no diarrhea). Specimen collected the 8th day.	3	4.6	.1	10	10	.0001	Green streptococcus and L. acidophilus
2/16	Regular diet for 13 days	3	7.0	100	1000	10	.00001	Green pleomorphic streptococci
2/27	2 cc. 50% lactose injected into ileum once every 15 minutes for 3 hours each day for 7 days. Specimen collected 8th day. ³	2	7.0	100	10	.001	Not found ¹	Bact. coli non-hemolytic
3/8	Fed 11 grams of lactose in bread and milk daily for 8 days. ⁴	2	5.0	10	1000	100	1	Green diplostreptococcus
Monkey C (cecal fistula)								
1933								
1/13	Fed regular meal	4	6.0	.001	10	Not tested	Not tested	Green pleomorphic streptococcus
1/20	Fed 300 cc. of autoclaved liver daily for 8 days (diarrhea). Specimen collected 8th day.	2	6.5	1	<1	Not found ¹	100 ⁵	Gram positive anaerobic rod (not Cl. welchii)
1/27	Regular diet for 8 days (stools well formed).	3	7.0	.1	<1	10	100	Gram positive anaerobic rod (not Cl. welchii)
2/3	Fed 50 grams lactose daily for 8 days (no diarrhea). Specimen collected 8th day.	3	4.6	Not found ¹	100	100	.01	L. acidophilus. Green diplostreptococcus
2/16	Regular diet for 13 days	3	5.6	.001	<10	10	.01	L. acidophilus
2/27	2 cc. 50% lactose injected into cecum once every 15 minutes for 3 hours each day for 7 days. Fecal specimen collected 8th day. ⁶	2	7.0	.001	<1	10	.01	L. acidophilus
3/8	Fed 11 grams of lactose in bread and milk daily for 8 days. ³	2	6.0	.001	<.1	1	.0001	L. acidophilus. Proteus-like organism.

¹ Not found in 0.01 cc. of specimen.² This organism was seen in smears. It did not grow on subculture either aerobically or anaerobically.³ In feces collected at the same time the relationships of Bact. coli, streptococci and L. acidophilus were practically the same as in the specimen from the higher level.⁴ In feces collected at the same time L. acidophilus was the predominating organism (10 times more numerous than Bact. coli).⁵ Cl. welchii was present 10 million per cc.⁶ No material could be aspirated from the cecum.

the stools. Such an increase was explained by some workers as due to the ingestion of large numbers of spores in the food, and by others to the growth stimulating action of carbohydrates in the diet. The diarrhea which the monkeys developed on the liver diet may have been due to the bile contained in the liver, since intestinal motility is increased by bile. No experiments were carried out to determine the cause of the diarrhea.

Lactose, fed in large and small quantities to these animals at different times, stimulated the growth of *L. acidophilus*. However, the daily injection of lactose into the ileum (monkey I) failed to increase the numbers of *L. acidophilus*. This is in agreement with the results obtained in dogs, where lactose was injected intracecally. The change to an aciduric flora by feeding lactose and the failure to obtain such a flora by injecting this carbohydrate into the lower levels of the intestine, indicates that in the former case lactose must undergo some change during digestion in the higher levels of the intestine.

The change in flora following lactose feeding may be due to unabsorbed acids resulting from the hydrolysis of lactose. Cannon and McNease,¹³ working with white rats on different diets, found that the reaction of the contents of the cecum of animals on a high protein diet was p_H 7.0 to 7.1, while a p_H of 4.6 was obtained in the cecum of rats fed lactose. Hudson and Parr²⁷ in similar experiments found that the reaction of contents of the cecum of rats on a high lactose diet ranged from p_H 4.6 to 5.0, while that of animals on a meat diet and on a stock diet (carrots, bread, oats and water) ranged from p_H 6.7 to 7.0.

In the experiments reported here the p_H of specimens from the ascending colon of dogs ranged from 5.3 to 8.2, the majority having a p_H of 6.0 to 7.0. Material from the same level in monkeys had a similar range of hydrogen ion concentration, except when large amounts of lactose were fed, at which time the p_H was lowered to 4.6. The intracecal injections of dextrose, lactose or peptone did not cause any appreciable change in the reaction.

THE EFFECT OF THE ABSENCE OF BILE ON CERTAIN ORGANISMS IN THE ASCENDING COLON OF A DOG

The inhibitory action of bile on the growth of *Bact. coli* in vitro has been well established.²⁸ However, few studies have been made to determine whether this action is also effective in vivo. Balice²⁹ reported that when bile was truly lacking from the intestine there was an increase in intestinal bacteria. In order to evaluate the effect of the exclusion of bile on organisms in the ascending colon, the following operation was performed on a dog under ether anaesthesia.

The abdominal cavity was opened by a mid-line incision. The common bile duct was doubly ligated. A rubber catheter was then anchored into the gall bladder with a purse string suture. The omentum was wrapped about the

27. J. Infect. Dis. **34**: 621, 1924.

28. Jordan, E. O.; J. Infect. Dis. **12**: 326, 1913.

29. Polclinico **34**: 501, 1927.

catheter at the place where it entered the gall bladder. The catheter was then brought to the outside through a stab wound. The tip of the cecum was brought out through another stab wound. The tip of the cecum was brought out through another stab wound. An opening was made into the cecum several days after operation.

This animal was allowed to recover from the operation before specimens were examined. Two specimens of material from the ascending colon were cultured, one 9 days and the other 19 days after the operation. The dog died on the 20th day following operation.

There was no striking difference in the results obtained from the two examinations on specimens from this animal, as compared with those from normal dogs. The *Bact. coli* count was high in each case, but was exceeded the first time by an aerobic gram negative rod (not *Bact. coli*) and the second time by a green-producing streptococcus. The streptococcus was found in the 1:200 billion dilution, a count somewhat higher than was usually obtained for the predominating organism in the contents of the ascending colon of normal dogs.

ATTEMPTS TO ALTER BACTERIAL TYPES AND RELATIONSHIPS IN ISOLATED SEGMENTS OF LARGE INTESTINE

It has been pointed out that these isolated segments of bowel do not receive material from the small intestine and, therefore, they offer an opportunity for a simple analysis of factors which may influence the relationships of bacteria in the large intestine. In the experiments reported here nutritional factors have received particular attention. Monkey CP, with an isolated pouch including the cecum, ascending colon and part of the transverse colon, and monkey TC, with an isolated segment of transverse colon, were used.

In a previous study¹ in which these monkeys were used, *Bact. coli* was found to multiply in isolated segments of large intestine. Since both ends of the segments were open to the outside, there was ample opportunity for the introduction of organisms. However, outside contamination was not indicated, since ordinary saprophytes were not found in appreciable numbers in the content of the isolated colon.

The flora in isolated segments of colon was studied before and after the injection of food materials. The food which was chosen for injection into the segments was similar to the diet of the animals and consisted of 40 cc. of sterile bread and milk. The pouch of monkey CP was injected with this material and specimens were withdrawn for culture one-half hour and 12 hours later. The samples were diluted immediately after collection in sterile saline and 1 cc. of each was inoculated into tubes of lactose broth and dextrose beef heart medium (table 4). Green-producing pleomorphic streptococci were equal in numbers to *Bact. coli* in the first specimen, but in the second they outnumbered *Bact. coli* 100:1. The bread and milk seemed to favor the growth of streptococci which were already present in large numbers.

Foreign organisms which enter the intestine with food or water are not

TABLE 4.—*Effect of the Injection of Bread and Milk and Lactose on the Bacterial Flora of Isolated Segments of Large Intestine of Monkeys*

Monkey CP					
Days after operation	Treatment	pH of specimen	Numbers in millions per cc. of specimen from intestinal loop		Predominating organism
			Bact. coli	Streptococci	
118	Pouch rinsed with 10 cc. of sterile saline	7.4	10	—	Gram negative rod 100 mill. (not Bact. coli)
147	Pouch rinsed with 10 cc. of sterile saline ¹	7.4	100	1	Bact. coli (hemolysis not tested)
180	Pouch rinsed with 10 cc. of sterile saline ^{1,2}	7.2	100	.001	Bact. coli hemolytic
75	40 cc. sterile bread and milk injected $\frac{1}{2}$ hour specimen	6.4	100	100	Green pleomorphic streptococci and Bact. coli (hemolysis not tested)
	12 hour specimen	5.4	1000	100,000	Green pleomorphic streptococci
93	30 cc. sterile bread and milk inoculated with Bact. prodigiosum (100,000 per cc.) injected $\frac{1}{2}$ hour specimen ³	6.4	100	—	Bact. coli non-hemolytic
	12 hour specimen ⁴	—	1000	—	Bact. coli non-hemolytic
100	10 cc. sterile bread and milk —50% lactose injected twice a day for 14 days. Specimen collected the 7th day	6.0	1000	1000	Green streptococci and Bact. coli non-hemolytic
107	Specimen collected the 14th day	6.0	100	1000	Pleomorphic streptococci non-hemolytic
191	2 cc. of 50% lactose injected once every 15 minutes for 3 hours each day for 7 days. Pouch rinsed with 5 cc. saline the 8th day ^{2,5}	4.8	1000	.01	Bact. coli hemolytic
Monkey CT					
37	Loop rinsed with 10 cc. of sterile saline	7.8	1	.1	Gram negative rod 10 mill. (not Bact. coli)
56	Loop rinsed with 10 cc. of sterile saline ¹	6.8	10	10	Bact. pyocyaneus 10,000 million ⁶
89	Loop rinsed with 10 cc. of sterile saline ^{1,2}	7.0	10	.01	Bact. coli hemolytic and non-hemolytic
19	15 cc. sterile bread and milk injected $\frac{1}{2}$ hour specimen	7.6	1000	—	Bact. coli (hemolysis not tested)
26	15 cc. sterile bread and milk injected $\frac{1}{2}$ hour specimen	6.8	100	—	Bact. coli non-hemolytic
12	30 cc. sterile bread and milk inoculated with Bact. prodigiosum (100,000 per cc.) injected $\frac{1}{2}$ hour specimen ⁴	6.4	100	—	Bact. coli non-hemolytic
	12 hour specimen ⁴	—	10,000	—	Bact. coli non-hemolytic
100	2 cc. of 50% lactose injected every 15 minutes for 3 hours each day for 7 days. 1 cc. of mucous aspirated the 8th day ^{2,5}	6.0	10,000	1	Bact. coli hemolytic

¹ Control experiments reported in previous paper.¹

² No *L. acidophilus* was found by direct plating in Torrey's medium nor in subcultures made in this medium from the dextrose beef heart cultures.

³ Bact. prodigiosum present 0.1 million per cc.

⁴ Bact. prodigiosum not found.

⁵ *L. acidophilus* was the predominating organism in the feces seven days before these cultures were made.

⁶ Bact. pyocyaneus wound infection at the time of operation.

— indicates that streptococci were not found.

found subsequently in large numbers in contents of the colon.³ This may be due to more rapid growth of intestinal organisms, to the inadequacy of present cultural methods or to the death of the exogenous bacteria. In vitro studies have shown that *Bact. prodigiosum* cannot be detected in the presence of *Bact. coli* when it is greatly outnumbered by the latter.¹ The isolated colon segments of two monkeys were used in an experiment in which an attempt was made to implant *Bact. prodigiosum*. Thirty cubic centimeters of sterilized bread and milk inoculated with *Bact. prodigiosum* were injected into the colonic pouches of both animals. Specimens were withdrawn and cultured one-half hour and 12 hours after injection (table 4). *Bact. coli* remained the predominating organism in both instances. *Bact. prodigiosum* was detected only once, in the one-half hour specimen from monkey CP.

Lactose fed in large amounts usually causes a predominance of *L. acidophilus* in the feces of man and animals. Two experiments were carried out to determine the effect of lactose on the flora of the isolated segments of colon. In the first, 10 cc. of sterile bread and milk containing 50 per cent lactose were injected into the pouch of the ascending and transverse colon (Monkey CP) daily for 14 days. Specimens were collected for culture on the 7th and 14th days, one-half hour after the injection of the pabulum. In both specimens streptococci were equal in numbers to *Bact. coli* (table 4). In the second experiment an attempt was made to simulate natural conditions by introducing small quantities of food into the colon at short intervals of time. Two cubic centimeters of 50 per cent lactose were injected into the isolated segments of colon of both monkeys once every 15 minutes for a period of 3 hours each day for 7 days. Specimens were collected the 8th day and cultured in lactose broth, dextrose beef heart and Torrey's mediums. *Bact. coli* was the predominating organism. The cultures in dextrose beef heart medium were subcultured in Torrey's medium, as well as on blood agar plates. No *L. acidophilus* was found (table 4). This observation is very interesting in view of the fact that cultures of feces from these animals, made 7 days previously, yielded *L. acidophilus* as the predominating organism, and undoubtedly there was ample opportunity for the contamination of the loop orifices with fecal organisms. However, it has not been demonstrated as yet whether *L. acidophilus*, if introduced experimentally, could survive and multiply in these segments of bowel.

The introduction of bread and milk or large amounts of lactose had little if any effect on the relationships of the bacteria in the isolated segments of large intestine. A foreign organism, such as *Bact. prodigiosum*, did not find conditions in the segments of colon favorable for its development.

DISCUSSION

The stability of bacterial relationships in the colon presents an important biological problem. At present there are no means of adequately studying this problem in vitro, since no test tube has been devised which can imitate the bowel. In the colon not only absorption but secretion and excretion of substances occur to complicate the nature of the problem. These changes are continuous and apparently maintain an equilibrium which is especially suited to certain microorganisms.

Chemical methods are not available whereby the course of many nutritional substances can be followed in the large intestine. The colon is always adequately provided with enormous numbers of different kinds of bacteria, and the activity of the enzymes elaborated by such bacteria only adds confusion to chemical studies.

Bact. coli was abundant in isolated segments of colon where the fecal stream, including secretions from higher levels, was absent. This organism is capable of maintaining itself in the absence of materials from the small intestine. Organisms other than *Bact. coli* and possibly streptococci are often found in large numbers in this region, and probably depend on the fecal stream for their nutrition. The chemical composition of this material, which is largely dependent upon the character of the diet, plays an important part in determining the flora. However, radical changes in the diet are necessary to effect appreciably the relationships of bacteria. A simplification in the flora (to principally *Bact. coli* and streptococci) occurred in segments of large bowel isolated from the rest of the intestinal tract. The injection of lactose in bread and milk or lactose alone into isolated colon segments did not change the relative types or numbers of organisms from those found when these segments were empty. The sterile food materials introduced into these segments were always heavily inoculated with billions of *Bact. coli* whose mere numbers have given this organism a decided advantage over all others.

Bact. coli under normal conditions in the large intestine is frequently outnumbered by other bacteria, especially cocci. The feeding of lactose often causes a predominance of the gram positive aciduric organisms accompanied by an increased acidity in the cecum. Whether acidity alone can bring about such a change in flora is not known, as there may be other products in lactose digestion which favor the aciduric organisms.

The intracecal injection of peptone, dextrose, lactose and bile apparently did not change the relationships of *Bact. coli* to other organisms in the ascending colon. Certainly these materials were introduced in sufficient quantity and at frequent enough intervals to simulate the natural flow of small bowel contents into the cecum during digestion. Since the fate of these substances in the large bowel is not understood, it is not known how long these substances were available to the bacteria.

SUMMARY

Attempts have been made to alter the relationships of bacteria in the colon and in isolated segments of large intestine by changing the nutritional environment. Three dogs and one monkey with cecal fistulae, one monkey with an ileal fistula, two monkeys with isolated segments of large intestine, and one dog with a biliary fistula were used in this study. The organism which outnumbered all others in the intestinal contents (as determined by methods used in these experiments) was isolated and compared with the numbers of *Bact. coli*, and in some experiments with the numbers of streptococci, *L. acidophilus* and spore-forming anaerobes.

The bacterial relationships in the ascending colon of dogs and monkeys were quite constant and were not appreciably altered during the digestive period by the incoming material from the small intestine.

The intracecal injection of dextrose, lactose, peptone and bile (separately) was without noticeable effect on the numbers and relationships of organisms present in the colon. Feeding an exclusive liver diet to two monkeys caused a great increase in the numbers of sporulating anaerobes in the intestinal contents of one of the animals. The oral administration of lactose markedly stimulated the growth of *L. acidophilus* in the large intestine of these monkeys.

The exclusion of bile from the intestine of a dog did not affect the flora of the large bowel.

The flora of isolated segments of colon in two monkeys was not significantly changed by the injection of bread and milk or lactose into these segments.

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